ULTRASTRUCTURE OF EOCENE FOSSIL PLANTS

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Abstract.— Petrified leaf and pericarpic tissues from the Eocene Deccan Intertrappean beds of India were studied using light and transmission electron microscopy. Degradated cytoplasm with organellelike bodies are present in cells of the leaf tissues. TEM of these cells revealed wall structure and cytoplasmic residues. Microfibril distribution of pericarpic cells resembles fiber cells in extant angiosperms.

Scanning and transmission electron microscopes are widely used in studying fossil plant material. Chaloner and Collinson (1975) demonstrated that additional information on impression fossils could be obtained by using SEM. Grierson (1976), Hartman (1977), and others have studied SEM structures of the vascular tissues of Devonian plant fossils. Several workers, notably Kedves (1974), Pettit (1966), Taylor (1968, 1973a, 1973b), Taylor and Millay (1969, 1977a), and others have reviewed the usefulness of scanning and transmission electron microscopy in paleopalynological investigations.

It was Wesley and Kuyper (1951) who, for the first time, demonstrated that TEM could be used in studying the vascular tissues of *Lepidodendron*. Since then, Eicke (1954), Fry (1954), and Schmid (1967) have employed TEM in studying vascular tissues of fossil plants.

Crepet et al. (1975) and Dilcher (1974) extended the electron microscopy studies to fossil angiospermic leaves and inflorescences. Niklas et al. (1978) described TEM structures of Miocene angiospermic leaves resembling *Zelkova* from the Succor Creek Formation of Oregon. Apart from these, the authors are unaware of any other TEM studies on petrified angiospermic tissues. Therefore, two different plant tissues from the Eocene Deccan Intertrappean series of India were processed for ultrastructural studies. These Intertrappean beds contain some of the best preserved petrifactions from India, and until now no electron microscope studies of the Deccan fossils have been attempted. Hence, the present work forms a unique attempt at describing the ultrastructure of these fossil plant tissues.

Material and Methods

The petrified fossil tissues are embedded in black cherts. Peel transfers of these fossil tissues were cut into 1–2 mm pieces. They were then placed in 1 percent OsO_4 for four to six hours. The sample pieces were transferred from OsO_4 and embedded in agar (2 percent/H_2O), to prevent disintegration in embedding solvents. The material was then allowed to remain in Uranyl acetate overnight. After dehydration by processing through a series of ETOH and acetone concentrations, the material was embedded in Mollenhauer's resin (1964). Ultrathin sections were subsequently prepared.

Results and discussions

Leaf Tissues

Several well-preserved petrified leaf tissues were embedded in a black chert. Epidermal cells of the leaf tissue are rectangular (Fig. 2) and many of these cells contain cytoplasm and organellelike bodies (Figs. 2 to 4) representing various degrees of disintegration. From their consistent peripheral orientation, the round to oval organellelike structures (Fig. 2) are similar to the distribution of chloroplasts in extant angiospermic leaves. A

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Fig. 1. Light micrograph of the epidermal cells of the fossil leaf tissue, 50X.

Fig. 2. One of the epidermal cells from the petrified leaf tissue enlarged (light micrograph). Arrows point out the round to oval organellelike bodies, 850X.

Fig. 3. A single cell of the Eocene leaf tissue, demonstrating its internal structure (light micrograph), 750X.

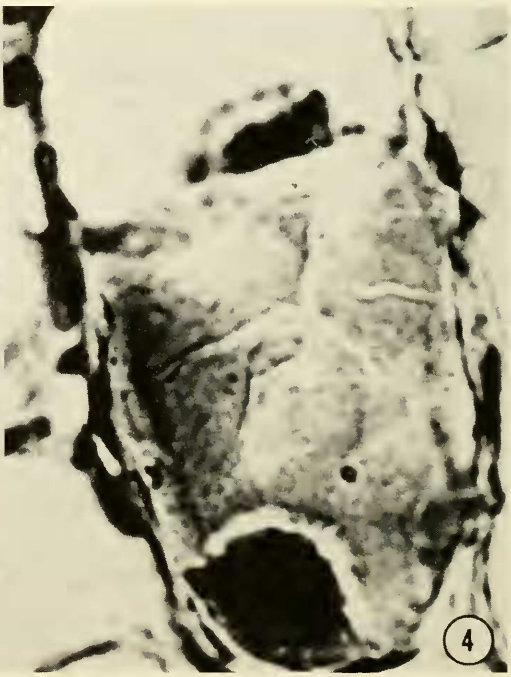

Fig. 4. Cell of the fossil leaf, showing degradated cytoplasm (light micrograph), 1300X.

Fig. 5. Structure of cell wall: middle lamella, isotropic layer, and secondary wall are visible (electron micrograph), 13,000X.

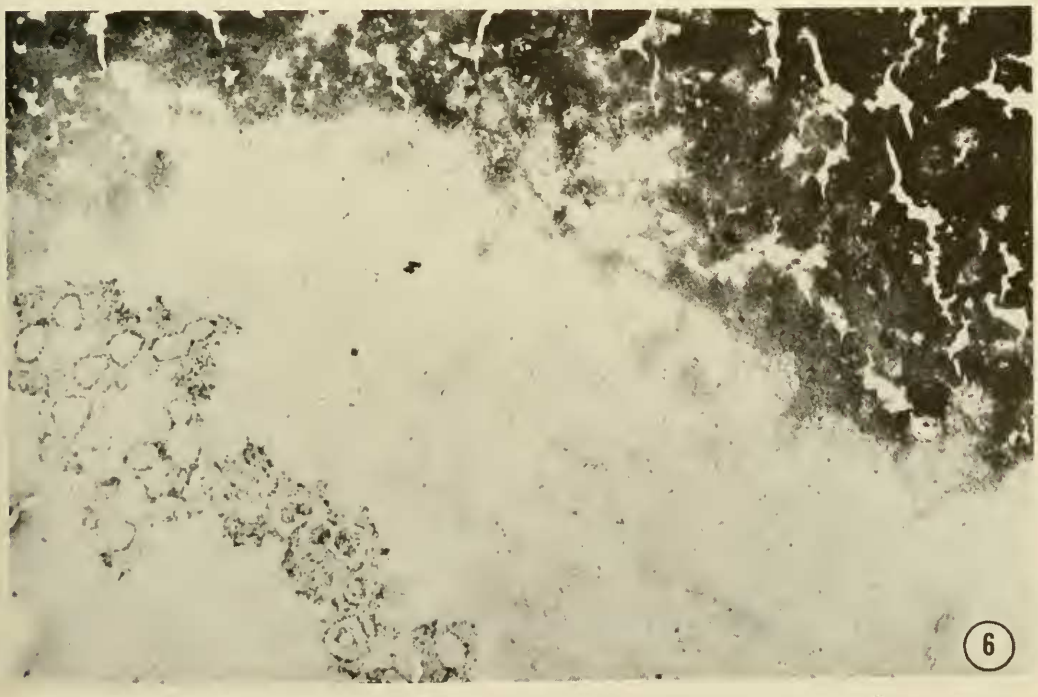

Fig. 6. Cytoplasmic residues inside the cells of the fossil leaf tissue (electron micrograph), 28,800X.

region thought to represent cytoplasm (Figs. 3 to 4) has undergone considerable degradation (Fig. 4), and whether a nucleus is preserved or not is a matter of conjecture.

The presence of protoplasmic material in fossil plant tissues is well documented by several workers. Taylor and Millay (1977a) suggested that cytoplasm and nuclei could be preserved in the microspores of the Pennsylvanian cone genus, *Lasiostrobus*. They later showed evidence for well-preserved cytoplasmic material in *Biscalitheca* spores (Taylor and Millay 1977b). In *Lepidostrobus schopfii* (Brack-Hanes and Vaughn, 1978) and *Zelkova* (Niklas et al. 1978), nuclear material and cytoplasmic residues have been reported. Chlorophyll derivatives were recovered from the Eocene formations of East Germany (Dilcher et al. 1970) and Miocene *Zelkova* leaves (Niklas and Giannasi, 1977). Baxter (1964) reported starch grains in Pennsylvania fossil gametophytes. The presence of protoplasts, although somewhat degradated, in the petrified leaf remains from the Deccan Intertrappean beds, therefore, adds another example of the preservation of cytoplasm and organellelike bodies in plant fossils. Relatively low degradation and, thus, the preservation of cytoplasm and organellelike bodies may have resulted from a rapid deposition in the numerous lakes that occupied the Deccan region in the Eocene period.

Cells of the leaf tissues, on the other hand, retain a wall structure (Fig. 5) that resembles parenchymatous cells in extant angiosperms. Two electron-dense layers are apparent with an isotropic layer between them. At the center, where cell walls meet, the middle lamella attains maximum thickness. The middle lamella is an electron dense layer, and represents a compound middle lamella as in the fossil genus, *Callixylon* (Schmid 1967). Several extant angiosperms such as species of *Populus* (Chafé and Chauret 1974) have similar middle lamellae. The inner electron dense layer corresponds to the inner secondary wall. Thickness of the isotropic layer (Fig. 5) is, perhaps, exaggerated by shearing of the material. S1, S2, and S3 layers, as found in lignified tissues (Schmid 1967), are indistinguishable in this fossil material. Cytoplasm has undergone considerable degradation. Cytoplasmic residues are found adjacent to the cell walls (Fig. 6). An electron-dense organic matter is occasionally present in the cytoplasmic residue (Fig. 7). The electron-dense nature of this material is perhaps due to the unsaturated bonds that have reacted with the osmic acid and probably is not from the coalification process. These are evidently not artifacts, but may or may not represent part of the cytoplasm.

Pericarpic Tissues

The pericarpic tissues processed for TEM studies are from peel transfers of *Viracarpon* Sahni, a monocot fruit of Pandanaceous affinities (Nambudiri and Tidwell 1978). The mesocarp of the phallanges in *Viracarpon* is formed of thick walled parenchymatous and fibrous tissues. The parenchymatous cells are moderately thick walled and isodiametric. The sclereids, on the other hand, are highly thick walled brachysclereids. The electron micrographs (Figs. 8 and 9) are of structures similar to the secondary wall layers of fiber cells. The microfibril distribution in the pericarpic cells (Fig. 9) suggests similarities with the gelatinous fibers of many extant plants such as *Celtis* sp., *Acer saccharum* and *Populus* sp. (Côté and Day, 1962). These authors observed a gelatinous layer around the secondary walls. The longitudinal splits that traverse the secondary walls (Fig. 2) could correspond to the terminal lamellae reported for the tension wood fibers in *Populus* (Côté and Day 1962).

From this brief TEM study of the leaf and pericarpic tissues from the Deccan Intertrappean beds of India, it is evident that the application of electron microscopy to supplement the anatomical data would further enhance closer comparisons with extant genera of angiosperms.

Acknowledgments

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Literature Cited

BAXTER, R. W. 1964. Paleozoic starch in fossil seeds from Kansas coal balls. Trans Kansas Acad. Sci. 67:418–422.

Fig. 7. Electron dense carbonaceous matter inside the cells of the fossil leaf tissue (electron micrograph), 13,000X.

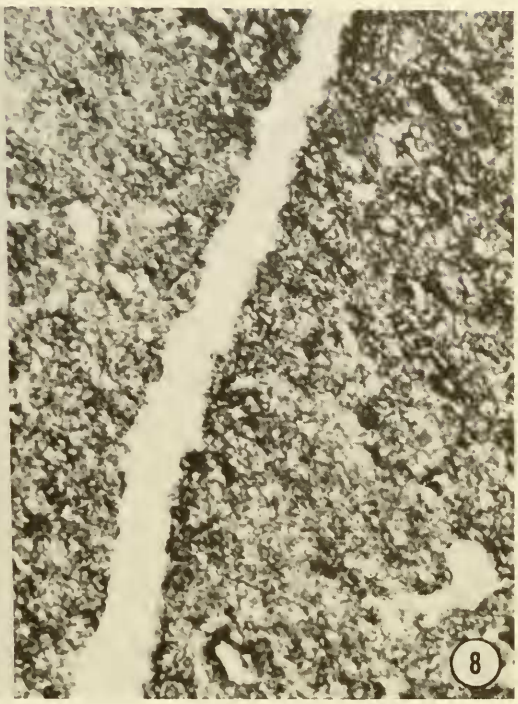

Fig. 8. Pericarpic tissue of *Viracarpon*, illustrating the lamella (electron micrograph), 28,800X.

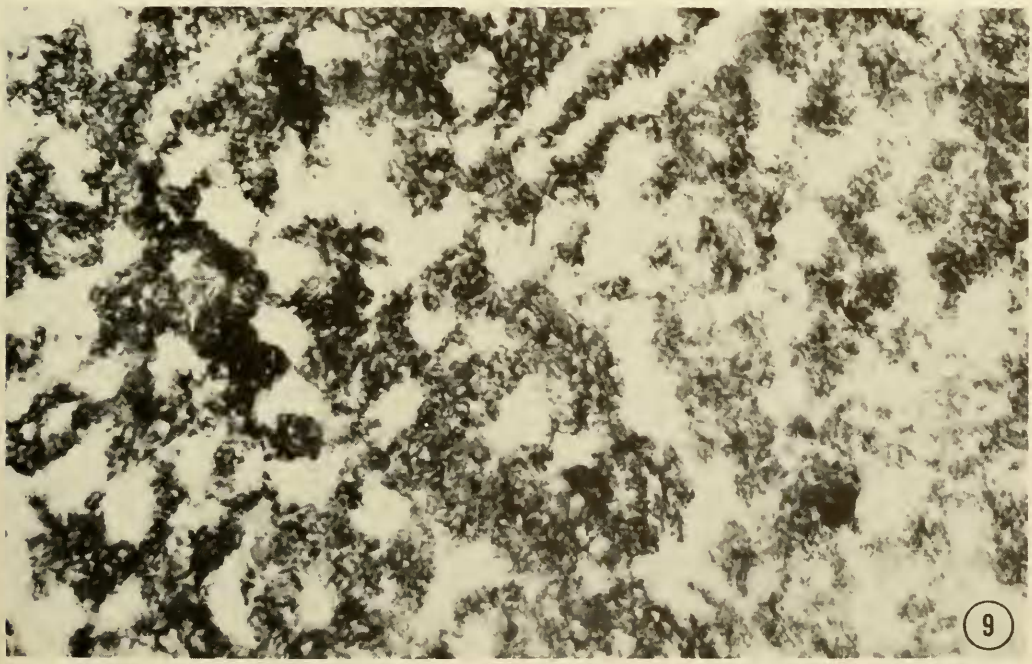

Fig. 9. Electron micrograph of pericarpic cells of *Viracarpon*, showing microfibril distribution, 28,800X.

BRACK-HANES, S. D., AND J. C. VAUGHN. 1978. Evidence of Paleozoic chromosomes from lycopod microgametophytes. Science 200:1383–1385.

CHAFÉ, S. C., AND G. CHAURET. 1974. Cell wall structure in the xylem parenchyma of trembling aspen. Protoplasma 80:129–147.

CHALONER, W. G., AND M. E. COLLINSON. 1975. Application of SEM to a sigillarian impression fossil. Rev. Palaeobot. Palynol. 20:85–101.

CÔTÉ, W. A., AND A. C. DAY. 1962. The G-layer in gelatinous fibers; electron microscopic studies. Forest Prod. J. 12:333–338.

CREPET, W. L., D. L. DILCHER, AND F. W. POTTER. 1975. Investigations of angiosperms from the Eocene of North America. A catkin with juglandaceous affinities. Amer. J. Bot. 62:813–823.

DILCHER, D. L. 1974. Approaches to the identification of angiosperm leaf remains. Bot. Rev. 40:1–158.

DILCHER, D. L., R. J. PAVLICK, AND J. MITCHELL. 1970. Chlorophyll derivatives in Middle Eocene sediments. Science 168:1447–1449.

EHRLICH, H. G., AND J. W. HALL. 1959. The ultrastructure of Eocene pollen. Grana Polynol. 2:32–35.

EICKE, R. 1954. Elektronenmikroskopische untersuchungen an verkielselten coniferen. Palaeontographica 97B:36–46.

FRY, W. L. 1954. A study of the carboniferous lycopod, *Paurodendron*, gen. nov. Amer. J. Bot. 41:415–428.

GRIERSON, J. D. 1976. *Leclercqia complexa* (Lycopsida, Middle Devonian): Its anatomy, and the interpretation of pyrite petrifactions. Amer. J. Bot. 63:1184–1202.

HARTMAN, C. M. 1977. Wall structure of tracheids of *Psilophyton dawsonii*. Bot. Soc. Amer. Misc. Series 154:38.

KEDVES, M. 1974. Elektronmokroszkopos vizsgalatok fosszilis zarvatermo pollenszemeken. Bot. Koxlem 61:283–287.

MOLLENHAUER, H. H. 1964. Plastic embedding mixtures for use in electron microscopy. Stain Tech. 39:111–114.

NAMBUDIRI, E. M. V., AND W. D. TIDWELL. 1978. On probable affinities of *Viracarpon* Sahni from the Deccan Intertrappean flora of India. Palaeontographica 166B:30–43.

NIKLAS, K. J., AND D. E. GIANNASI. 1977. Flavanoids and other chemical constituents of fossil Miocene *Zelkova* (Ulmaceae). Science 196:877–878.

NIKLAS, K. J., R. M. BROWN, R. SANTOS, AND B. VIAN. 1978. Ultrastructure and cytochemistry of Miocene angiosperm leaf tissues. Proc. Natl. Acad. Sci. U.S.A. 75:3263–3267.

PETTIT, J. M. 1966. Exine structure in some fossil and recent spores and pollen as revealed by light and electron microscopy. Bull. British Mus. Natur. Hist. Geol. 13:221–257.

SCHMID, R. 1967. Electron microscopy of wood of *Callixylon* and *Cordaites*. Amer. J. Bot. 54:720–729.

TAYLOR, T. N. 1968. Application of scanning electron microscopy in paleobotany. Trans. Amer. Microsc. Soc. 87:510–515.

———. 1973a. A consideration of morphology, ultrastructure, and multicellular microgametophyte of *Cycadeoidea dacotensis* pollen. Rev. Palaeobot. Palynol. 16:157–164.

———. 1973b. Combined transmission and scanning electron microscopy of *in situ* Palaeozoic spores. Palaeontology 16:157–164.

TAYLOR, T. N., AND M. A. MILLAY. 1969. Application of the scanning electron microscope in paleobotany. Proc. 2d Annual SEM Symp.: 105–115.

———. 1977a. The ultrastructure and reproductive significance of *Lasiostrobus* microspores. Rev. Palaeobot. Palynol. 23:129–137.

———. 1977b. Structurally preserved fossil cell contents. Trans. Amer. Microsc. Soc. 96:390–393.

WESLEY, A., AND B. KUYPER. 1951. Electron microscope observations on the xylem elements of a fossil plant. Nature 168:137–140.